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Clinical-pathological Correlations in Post-Transplant Thrombotic Microangiopathy

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Conflict of Interest Statement

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Abstract

Aims

Post-transplant thrombotic microangiopathy (TMA) is a rare and clinically challenging finding in renal transplant biopsies. In addition to recurrent atypical haemolytic uremic syndrome (aHUS), TMA in renal transplants is associated with various conditions, such as calcineurin-inhibitor (CNI) treatment, antibody-mediated rejection (ABMR), viral infections, sepsis, pregnancy, malignancies or surgery. The therapeutic implications of this diagnosis are considerable. In order to better understand post-transplant TMA and to identify histological or clinical

differences between associated causes, we conducted a multi-centre retrospective study.

Methods and Results

Clinical parameters and transplant renal biopsy findings from 81 patients with TMA were analysed. Biopsies from 38 patients were also analysed by electron microscopy. Based on clinical-pathological correlation, TMA was attributed to a main aetiology, whenever possible. TMA occurred at a median of 30 days post-transplantation. Systemic features of TMA were present in only 18%. 22% of cases were attributed to CNI and 11% to ABMR. Although other potentially contributing factors were found in 56% of patients, in most cases (63%) no clearly attributable cause of TMA was identified. Histological differences between groups were minimal. Detection of ultrastructural features usually associated with ABMR may help establish ABMR as the cause of TMA.

Conclusions

Although CNI and ABMR appear to be the main contributors to post-transplant TMA, aetiology of most cases is likely multifactorial and TMA cannot be unequivocally attributed to a single underlying aetiology. Morphological features of TMA are not discriminating but electron microscopy may help identify ABMR-associated TMA.

Key words

Thrombotic microangiopathy, kidney transplantation, antibody mediated rejection, calcineurin inhibitor, electron microscopy

Introduction

Thrombotic microangiopathy (TMA) is a condition in which there is endothelial damage in small vessels due to a variety of aetiologies, resulting in thrombosis. The kidney is often affected. The clinical picture associated with TMA varies and may or may not present with the classical triad of haemolytic uremic syndrome (HUS) including thrombocytopenia, haemolytic anaemia and renal dysfunction. Shiga-toxin producing *E.coli* and *S.pneumoniae* associated HUS account for most of the typical forms of HUS, and for the great majority of HUS in chil-

dren. Atypical HUS (aHUS) results from inherited or acquired dysregulation of the alternative pathway of complement activation (reviewed in (1)). Thrombotic thrombocytopenic purpura (TTP) represents the third main clinical condition associated with TMA, caused by deficiency of ADAMTS13. There are many other potential causes of TMA, and, to complicate the picture, the morphological findings also vary: Some cases show acute thrombotic changes while chronic microangiopathic lesions dominate in others (2).

TMA in renal transplant biopsies reflects either recurrence of the underlying disease or a *de novo* complication. *De novo* TMA is seen in 0.8% to 14% of renal transplant biopsies (3, 4). Various extrinsic risk factors, which potentially injure the endothelium and thereby trigger TMA, are found in kidney transplant recipients. Many studies focus on calcineurin-inhibitor (CNI) treatment as a cause of post-transplant TMA (4-11) while more recently antibody-mediated rejection (ABMR) has been considered to be the main trigger for TMA in some studies (12, 13).

In some cases however, glomerular and/or arteriolar thrombi are seen in the absence of any other characteristic histological features of ABMR thus it is unclear whether these cases are related to ABMR, CNI toxicity, recurrent disease or other known aetiologies of TMA. Clinical diagnosis is further complicated by the fact that the classical triad of HUS is often lacking in post-transplant TMA (12).

In the absence of microvascular inflammation, a diagnosis of ABMR-related TMA requires the presence of 2 of the 3 following features: C4d-positivity, donor-specific antibodies (DSA) or increased expression of ABMR-related transcripts (14). C4d as a diagnostic marker, however, is limited by its low sensitivity (15), and validated transcript-analysis is not widely available. Previous studies on small numbers of patients suggested that isolated glomerular thrombi without arteriolar involvement may help identify ABMR-related TMA, but significant morphological overlap with other causes of TMA was recorded (12, 16).

Identifying the underlying cause of TMA in renal transplant biopsies can be particularly challenging yet the therapeutic implications are considerable. The Banff Working Group for TMA has called for more studies to address the morphological features of TMA in various settings

(14). The aim of this study was to give a comprehensive picture of TMA in renal transplant patients and to identify underlying causes based on retrospective analysis and careful correlation of pathological and clinical data. Here, we present the largest multi-centre study to date on post-transplant TMA, including 264 biopsies from 81 patients.

Methods

Study design and data collection

Four UK transplant centres contributed to this retrospective study. The study met approval from the National Research Ethics Service (REC reference 14/EM/1245; 10 Nov 2014) and from all site-specific Departments of Research and Development.

Histopathology electronic archives were searched for kidney transplant biopsies showing TMA, using search terms "thrombotic microangiopathy", “thrombosis“, “thrombus“. For the purposes of this study we specifically selected cases showing acute thrombotic microangiopathy, regardless of co-existing chronic/ non-thrombotic lesions. Thus, all cases showed thrombus, defined as presence of thrombotic material (platelet and/or fibrin) within glomerular capillaries, arterioles or arteries (figure 1). The first biopsy showing TMA is referred to as the index-biopsy. Banff lesion scores, result of C4d stain, main biopsy diagnoses and the following characteristics of TMA were recorded: Presence or absence of thrombi in glomeruli, arterioles and arteries; proportion of affected glomeruli and segmental or global extent of TMA in glomeruli. In addition, presence or absence of the following non-thrombotic lesions was assessed: mesangiolysis, fragmented red blood cells, collapse or bloodless appearance in glomeruli; intimal oedema, fragmented red blood cells and intramural fibrin in arterioles and arteries as well as presence or absence of onion skin lesions and fibrous intimal thickening without elastosis in arteries. Main diagnosis for previous or subsequent biopsies and presence of TMA in subsequent biopsies was also recorded. In total, 81 patients with 264 transplant kidney biopsies were included (figure 2). Baseline clinical variables are listed in table 1 and clinical variables related to the index-biopsy in table 2.

Clinical-pathological classification of TMA cases

Based on retrospective integration of clinical and histological parameters we assigned the most likely cause of TMA to every case if possible: Antibody-mediated rejection related TMA (ABMR-TMA) required positive donor-specific anti-HLA antibodies (DSA) at the time of index-biopsy, plus either capillaritis+glomerulitis>1, C4d>1 (excluding ABO-incompatible cases), or Banff v3 transmural arteritis. Cases with high CNI-level at the time of index-biopsy and histologically C4d negative TMA with no other features of ABMR, and no other obvious reason for TMA were designated as CNI-related TMA (CNI-TMA). Definition of high CNI-level was subject to local policy. Cases with other clear causes for TMA were designated OTHER-TMA. All other cases were classified as uncertain (UNCERTAIN-TMA).

Electron microscopy

Electron microscopy (EM) results were available for multiple biopsies from 38 patients, not necessarily corresponding to the index-biopsy. The following EM parameters, addressing signs of both acute and chronic endothelial damage, were compared between TMA groups: Mean and maximum number of peritubular capillary (ptc) basement membrane layers in at least 10 examined ptc (48 and 49 biopsies, respectively), presence of glomerular basement membrane double contours (44 biopsies), glomerular endothelial swelling (42 biopsies), lamina rara interna expansion (46 biopsies), presence of intracapillary cells in glomeruli (39 biopsies) and loss of glomerular endothelial cell fenestration (25 biopsies).

Statistics

Data were collected and analysed with STATA® version 14. Categorical data are given as absolute numbers and percentage and were compared using Fisher's exact test. Continuous variables in tables are expressed as mean ± standard-deviation or median and interquartile range for skewed data. Continuous variables were compared using Wilcoxon or Kruskal Wallis test and post hoc analysis with Dunn's test and Bonferroni correction of p-values for multiple pairwise comparisons for not normally distributed variables. P-values <0.05 were

regarded statistically significant. Prism® version 7 was used for graphic presentations.

Results

Patient characteristics

There were 81 patients with post-transplant TMA in the study with clinical parameters shown in table 1 (baseline data) and table 2 (related to the index-biopsy). Selected Banff lesion scores as well as the main histological diagnosis in index-biopsies are shown in table 3. Patients were transplanted between 2003 and 2014 with a median follow up of 669 days. Mean age at transplantation was 45 years, 85% received their first kidney transplant. 14% had pre-existing DSA. 31% of patients lost their graft within the follow up period and 4% died with a functioning graft. At the time of index-biopsy 17% of patients had non donor-specific anti-HLA antibodies, 23% had DSA.

The primary renal disease was frequently unknown (21%) and nephrosclerosis/hypertension as underlying renal disease was recorded in 5% of patients. Atypical HUS was clinically assumed in one patient; genetic testing did not show clearly known pathologic mutations in CFI, CFH, CD46, C3 and CFB genes but heterozygosity for a variant of uncertain significance in the CFI gene.

Characteristics of TMA

25% of patients had TMA in more than one biopsy. Signs of systemic TMA (defined as presence of both schistocytes and thrombocytopenia) were seen in 18% of patients at the time of presentation: Schistocytes were present in 18% of patients, drop in platelets was seen in 33%. Median time to diagnosis of TMA post-transplant was 30 days (IQR 9-250), 76% of patients had TMA within the first year. The time between transplantation and TMA diagnosis did not differ significantly between recipients of deceased, living ABO compatible, or living ABO incompatible transplants, nor did it correlate with the cold ischemia time. TMA was seen in 1.3 to 2.6% of all transplant biopsies within the study period at the different centres. Histological features of TMA are shown in table 4.

Clinical-pathological classification of TMA cases

Based on integration of clinical and histological parameters a single most likely aetiology could be assigned in 37% (30/81) of patients: TMA was attributed to ABMR in 11% (9) of patients (ABMR-TMA), all of which had DSA at the time of index-biopsy. TMA was attributed to CNI-toxicity in 22% (18) of patients (CNI-TMA), all of which had high CNI-levels at the time of index-biopsy (or immediately before biopsy in one patient). TMA was attributed to other clear causes in 3 patients (OTHER-TMA: hypertension-associated TMA, recurrent SLE with antiphospholipid syndrome and likely recurrent aHUS, respectively). In 63% (51) the aetiology of TMA remained uncertain (UNCERTAIN-TMA).

Additional contributing factors

For every patient the presence or absence of any further factors potentially contributing to TMA were recorded (excluding those factors which determined the assumed main aetiology of TMA in individual cases). 56% (45) of patients had one or more potentially contributing factor, none of which could be confidently identified as the main aetiological reason for TMA. Details are shown in figure 3.

Electron microscopy (EM)

EM was available for 38 patients and results are shown in figure 4. The limited number of EM results from the index-biopsies precluded sub-analysis of EM specimens from index-biopsies only and therefore includes even EM done on follow up biopsies. Due to small sample size and repeat biopsies from individual patients statistical analysis was not performed.

Comparison between TMA groups

Both TMA characteristics and clinical parameters were compared between four clinical-pathological TMA groups (tables 1, 2 and 4). The only significantly different parameter on histology was the extent of TMA in glomeruli which was mostly segmental in CNI-TMA (93%

cases segmental). Time from transplantation to index-biopsy was shortest in CNI-TMA and OTHER-TMA. By definition, ABMR-TMA had more DSA at biopsy and CNI-TMA had high CNI levels at biopsy. Neither mean GFR at index-biopsy nor at follow up were different between groups. The proportion of graft losses was highest in ABMR-TMA but did not significantly differ between groups.

We also limited the analysis to comparison between ABMR-TMA and CNI-TMA. Histologically, the extent of TMA in glomeruli was significantly different (less frequently global involvement of glomeruli in CNI-TMA). Also, mesangiolysis and fragmented red blood cells in glomeruli were significantly more frequent in ABMR-TMA. Clinically, CNI-TMA cases less frequently had a previous kidney transplant or anti-HLA antibodies prior to transplantation.

Focus on cases in which aetiology of TMA remained uncertain

In the majority of patients (51/81, 63%) no unequivocal underlying aetiology could be determined (UNCERTAIN-TMA). In 37 patients within this group one or more potentially contributing factors, as described above, were present (figure 3). Of note, 10 patients within this group showed morphological features of ABMR in their index-bx (either g+ptc>0 and C4d positive or g+ptc>1 and C4d negative plus Banff v3 lesion score in 2 patients) but did not have DSA. Nine more patients had DSA but neither microvascular inflammation nor C4d-positivity (or C4d-positivity in the context of ABOi transplantation, precluding its use to assign cases to ABMR). Together, these 19 cases showed some but not all features of ABMR so were not formally assigned to the ABMR-TMA category.

Electron microscopy was available for 26 patients (38 biopsies) from the UNCERTAIN-TMA group, not necessarily taken at the time of index-biopsy. In order to simplify assessment of ultrastructural changes in this group we combined five dichotomous EM-parameters (glomerular endothelial swelling, lamina rara interna expansion, loss of endothelial fenestration, presence of glomerular double contours and presence of intra-capillary cells in glomeruli) if available, to a score: no EM changes; mild: changes in 1-2 parameters; moderate: changes in 3 parameters; severe: changes in 4-5 parameters.

Of 4 cases that had high CNI levels at index-biopsy as a possibly contributing factor, none had more than mild EM-changes. 3 of 4 cases with a morphological picture within the ABMR-spectrum (but without evidence of DSA) had moderate or severe EM-changes. Presence of DSA at the time of index-biopsy was not per se associated with more severe EM changes in this subgroup (4/6 cases with DSA at index-biopsy had only mild EM changes). In cases for which no contributing factor was identified and EM was available (n=6), EM changes ranged from absent to severe without any discernible pattern.

Discussion

Thrombotic microangiopathy following kidney transplantation is a relatively rare microscopic finding with multiple aetiologies. Prevalence of *de novo* TMA after kidney transplantation varies considerably in the literature, ranging from 0.8 to 14% (3, 4). This might even be an underestimate as clinical manifestations, which could be a trigger for transplant biopsy, are not present in most cases. In addition, standardised histologic criteria for microangiopathic lesions are lacking. The clinical challenge of TMA in the transplant setting is the number of potential underlying causes with conflicting therapeutic implications. Integration of clinical, serological and histological findings therefore is paramount in order to ascertain a firm diagnosis.

Against this background we carried out a large, multi-centre retrospective study of clinical and morphological findings in post-transplant TMA. We limited our analysis to cases with frank thrombotic lesions and found that: TMA occurred at a median of 30 days post-transplantation in 2% of transplant kidney biopsies. Timing was not influenced by transplant type (living versus deceased) or cold ischemia time. Systemic features of TMA were only present in 18%. CNI-treatment accounted for 22% of cases and ABMR for 11%. Although potentially contributing factors were identified in overall 56% of patients, in most cases (63%) there was not one clearly attributable cause of TMA even after careful integration of histological and clinical features. Likely, ABMR does account for more cases but either typical morphological features or DSA are lacking, precluding a definite diagnosis. Histological

differences between causes were limited. Detection of features usually associated with ABMR by electron microscopy may help establish ABMR as the cause of TMA.

Our study concurs with previous reports in noting that de novo post-transplant TMA frequently occurs within the first months after transplantation (13, 17) and most often without systemic manifestations (12). A drop in platelet count was observed in 33% of patients, although in the post-transplant setting, plasmapheresis or immunosuppressive drugs in itself can reduce platelet count. Schistocytes were seen in 18% and a combination of both was observed in 18%.

Using strict criteria, CNI-treatment accounted for 23% of cases and ABMR for 11%. However, another 23% of patients had either DSA alone or morphological features of ABMR alone. In the literature, the proportion of TMA-cases attributed to ABMR is variable and represents up to 55% of cases (12, 13, 18). In this study we used strict and up-to-date criteria, which might explain our lower percentage. Particularly in these morphologically ambiguous cases, analysis of ABMR-associated gene transcripts may help identify cases related to ABMR.

Recurrent aHUS was suspected in one patient who did not have a clearly pathogenic mutation but a variant of uncertain significance in CFI gene. It is likely that some of the cases in this cohort may represent recurrent aHUS, where the cause of end-stage renal failure was either unknown or incorrect. Genetic testing for TMA susceptibility-genes was not widely available during the study period. Le Quintrec et al. found rare variants in complement regulatory genes (initially reported as pathogenic mutations) in 29% of patients with *de novo* post-transplant TMA, but not in a control group, suggesting that genetic variants may render a subset of patients susceptible to develop TMA in a post-transplant setting with increased endothelial stress for various reasons (1, 19).

We identified one or more risk factors which likely contributed to TMA in 56% of patients. In most of these cases, none of the contributing factors could be unequivocally assigned as the underlying cause, either because not all defined diagnostic criteria were fulfilled, or the picture was too complex to identify one clear aetiology. Whilst the presence of one risk factor may not have been significant, in the presence of an abnormal complement response to

stimuli, this may be sufficient to trigger a TMA response. These findings underscore the complexity in transplanted patients where many potential causes either in isolation or in combination can trigger TMA.

Comparison of light microscopic and clinical TMA-characteristics between ABMR- and CNI-related TMA revealed only minor differences. This is in keeping with KDIGO guidelines for native biopsies, which do not recommend attribution of a given cause to TMA on the basis of histological features alone (2).

Electron microscopy as an adjunct tool for early detection of ABMR has gained new attention recently (20-25). We and others have found that early endothelial changes such as endothelial swelling, subendothelial widening and loss of endothelial fenestration, are more frequent in biopsies with evidence of ABMR and/or DSA compared to ABMR/DSA-negative biopsies (24, 26). Haas and colleagues studied early ultrastructural changes in transplant renal biopsies with respect to ABMR. They infrequently found such changes in cases with CNI-toxicity in the absence of evidence of ABMR, and concluded that early ultrastructural glomerular changes seem to be relatively specific for ABMR (26). However, ultrastructural changes of CNI-toxicity have not been studied systematically. One may speculate that, in contrast to dose-dependent transient CNI-induced endothelial stress, DSA induce enduring endothelial damage and subsequently, structural remodeling of endothelium and basement membranes. Liapis et al. focused on peritubular capillary basement membrane multilayering in both native and transplant biopsies and found that chronic ABMR carried the highest risk for severe ptc multilayering although the latter was seen in native kidneys with chronic TMA (27). Here, we observed most pronounced endothelial and basement membrane changes in TMA cases attributed to ABMR. However, the mere presence of DSA was not per se associated with these changes. In conclusion, ultrastructural abnormalities largely reflected the light microscopy findings and severe ultrastructural changes argue against acute CNI-induced TMA. However, EM-results need to be interpreted with caution, as numbers of individual parameters in each group were small or incomplete. Transcript analysis and ultrastructural analysis may be useful tools in distinguishing between CNI- and ABMR-

induced TMA in cases where other diagnostic criteria fail to establish a clear diagnosis, and further investigation of both of these adjunct techniques would be worthwhile.

Overall, the present study confirms that light microscopic histopathology alone gives little indication of the causes of post-transplant TMA. Careful clinical-pathological correlation is crucial with a substantial proportion of cases that continue to remain unclear or complex and multifactorial. The addition of both functional and genetic testing for complement abnormalities may give insight into patients with TMA, even those who have an identified risk factor, in order to guide therapies, such as CNI avoidance in those more susceptible to endothelial dysregulation. Pathologists should provide a differential diagnosis of potential causes of TMA and emphasise that even identification of a potential trigger does not exclude an underlying genetic predisposition. Future studies in order to improve diagnosis and patient management should include complement genotyping, ultrastructural examination and transcript analysis for ABMR-associated gene expression.

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Authors' contributions

Verena Broecker: Conception and design of the study, analysis and interpretation of the data, writing the manuscript

Victoria Bardsley: Participated in performance of the research and revised the manuscript

Nicholas Torpey: Participated in performance of the research and revised the manuscript

Ranmith Pereira: Participated in performance of the research and revised the manuscript

Rosa Montero: Participated in performance of the research and revised the manuscript

Anthony Dorling: Participated in performance of the research and revised the manuscript

Andrew Bentall: Participated in performance of the research and data analysis and revised the manuscript

Desley Neil: Participated in performance of the research and revised the manuscript

Michelle Willicombe: Participated in performance of the research and revised the manuscript

Miriam Berry: Participated in conception and performance of the research, interpretation of the data and drafting the manuscript

Candice Roufosse: Participated in conception and performance of the research, interpretation of the data and drafting the manuscript

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Figure legends

Figure 1: Histological appearances of post-transplant TMA

A: Thrombotic material is seen in a glomerular segment extending from the hilus (Jone's Silver stain, original magnification x200). B: A glomerulus showing mesangiolysis with red blood cell extravasation (H&E, original magnification x200). C: Two small arterial branches showing subendothelial oedema and bloodless glomeruli (H&E, original magnifiaction x100). D: Subintimal oedema and red blood cell fragments are seen in a small artery (Jone's Silver stain, original magnification x200). E: Subendothelial red blood cell fragments are seen in an arteriole (H&E, original magnification x400). F: A small artery showing subendothelial oedema and fibrin (H&E, original magnification x200)

Figure 2: Patient cohort and biopsy material

The first biopsy showing TMA is defined as the patient's index biopsy. Transplant biopsies before and after the index biopsy within the study period were also recorded (non-index biopsies). 27 follow up biopsies showed TMA in addition to the index biopsy, which means that 41% of all biopsies showed TMA.

Figure 3: Contributing factors

In 56% of patients (45/81) one or more factors potentially contributing to TMA were found, which could not be assigned unequivocally as the main cause for TMA.

In 14 patients in the UNCERTAIN-TMA group no contributing factor was identified, thus in these 14/81 patients (17%) aetiology of TMA remained entirely unclear. Histologically, 13 of those cases showed TMA in their index biopsy without microvascular inflammation and one showed Banff I acute cellular rejection.

*Sum of numbers in parenthesis is higher than numbers of patients as a few cases have more than one contributing factor.

§Possible pro-thrombotic underlying conditions include history of scleroderma, history of malignant hypertension, history of detected Lupus anti-coagulant (but diabetes as underlying disease), history of SLE with Lupus anti-coagulant, history of antiphospholipid syndrome, donor history of scleroderma

$Other factors include Treatment for tuberculosis (in 2 cases), de novo post-infectious glomerulonephritis, acute CMV-infection, lung transplant, pancreatic surgery, sepsis, histiocytic glomerulopathy of uncertain aetiology

Figure 4: Ultrastructural changes in glomeruli and peritubular capillaries in different aetiological groups of TMA

Ultrastructural changes in glomeruli and peritubular capillaries are shown. Both glomerular basement membrane and glomerular endothelial changes as well as peritubular capillary basement membrane multilayering (ptcbml) are most prominent in ABMR-TMA. In contrast, ultrastructural changes are infrequent in CNI-TMA. The limited number of samples in each group and inclusion of subsequent biopsies from individual patients precluded statistical analysis. Electron microscopy in the subgroup of UNCERTAIN-TMA is described in more detail in the results-section.

GBM: Glomerular basement membrane; ptcbml: peritubular capillary basement membrane

multilayering. Total number of biopsies analysed in parenthesis.

*No cases available from CNI-TMA group for this analysis

Tables

Table 1: Patient details and baseline clinical data for all patients and compared between TMA-groups

			Assumed main etiological groups									
	All patients (n=81)	total number of valid observations¶	**ABMR-related TMA (n=9)**	total number of valid observations¶	**CNI-related TMA (n=18)**	total number of valid observations¶	**other assumed cause of TMA (n=3)**	total number of valid observations¶	**cause of TMA unclear (n=51)**	total number of valid observations¶	**p-value***	**p-value†**
Mean age at tx; y	45 ± 13	81	43 ± 6	9	40 ± 12	18	26 ± 9	3	49 ± 13	51	0.012	n.s.
Recipient gender; female/male	43%/57%	81	67%/ 33%	9	28%/ 72%	18	100%/ 0%	3	41%/ 59%	51	0.052	0.064
Mean donor age; y	48 ± 13	55	44 ± 12	4	48 ± 12	15	49	1	49 ± 15	35	n.s.	n.s.
Type of transplant		81		9		18		3		51	n.s.	0.061
deceased including pancreas/ kidney	52%		22%		50%		33%		59%			
living, ABOc	36%		56%		46%		67%		27%			

living ABOi	12%		22%		6%		0%		14%			
Re-transplanted	15%	79	50%	8	6%	18	50%	2	12%	51	0.011	0.02
Induction		79		8		18		2		51	0.001	0.000
Basilixi-mab	48%		0%		83%		100%		41%			
Campath	34%		50%		6%		0%		43%			
PIEx/Campath/ Rituximab	5%		25%		0%		0%		4%			
Basilixi-mab/Rituximab	4%		0%		6%		0%		4%			
other/unknown	9%		25%		6%		0%		8%			
Mean CIT; h	11.1 ± 7.2	60	9.4 ± 8.3	4	8.1 ± 6.4	17	8.3 ± 8.1	2	12.9 ± 7.4	37	n.s.	n.s.
Mean number of HLA-mismatches	3.1 ± 1.6	80	3.8 ± 1.5	9	2.9 ± 1.7	17	3 ± 0	3	3.1 ±1.7	51	n.s.	n.s.

Pre-existing anti-HLA anti-bodies		78		9		18		3		48	0.000	0.000
No antibodies	65%		11%		89%		100%		65%			
anti-HLA, non donor-specific	21%		11%		11%		0%		27%			
donor-specific	14%		78%		0%		0%		8%			
Original dis-ease		81		9		18		3		51	n.s.	n.s.
Glomeulo-nephritis	16%		11%		17%		33%		16%			
Vasculitis	5%		0%		6%		0%		6%			
SLE/APLS	6%		11%		0%		33%		6%			
Systemic scle-rosis	1%		0%		6%		0%		0%			
FSGS	4%		0%		11%		0%		2%			
Diabetes	14%		11%		0%		0%		20%			

Nephro-sclerosis/ hy-per-tension	5%		0%		6%		0%		6%			
CNI-toxicity	2%		0%		0 %		0%		4%			
Reflux/obstruc-tion/pyelonephri tis	9%		33%		17%		0%		2%			
Hereditary renal disease‡	17%		22%		22%		0%		16%			
Oth-er/unknown§	21%		11%		17%		33%		24%			

Percentage may not sum to 100 due to rounding

¶Total number of valid observations may vary from total number of cases within respective group due to missing values

Tx, transplantation; ABOc, Blood group ABO compatible; ABOi, Blood group ABO incompatible; bx, biopsy; PIEx, Plasma exchange; CIT, cold ischemia time; SLE, systemic lupus erythematosus; APLS, antiphospholipid syndrome; FSGS, focal segmental glomerulosclerosis; CNI, calcineurin inhibitor

*Comparison between all groups; † comparison between ABMR- and CNI-related TMA

‡ Alport's, cystic diseases and developmental kidney defects; § one of which with assumed but not genetically confirmed aHUS in childhood, one with end-stage kidney disease due to nephrocalcinosis, one with possible but unconfirmed systemic sclerosis, all other cases in this group unknown

Table 2: Clinical data related to the index-biopsy for all patients and compared between TMA-groups

			Assumed main etiological groups									
	All patients (n=81)	total number of valid observations¶	**ABMR-related TMA (n=9)**	total number of valid observations¶	**CNI-related TMA (n=18)**	total number of valid observations¶	**other assumed cause of TMA (n=3)**	total number of valid observations¶	**cause of TMA unclear (n=51)**	total number of valid observations¶	**p-value***	**p-value†**
Median time from tx to index bx; d (IQR)	30 (9-250)	81	17 (9-716)	9	10 (9-16)	18	7 (6-1186)	3	89 (17-411)	51	0.0096	n.s.
Median length of follow-up/IQR; d	669 (337-1121)	81	457 (47-1533)	9	955 (532-1656)	18	376 (30-730)	3	621 (243-1035)	51	n.s.	n.s.
Immuno-suppr.at index bx		78		9		17		2		50	n.s.	n.s.
MMF, Pred, CsA	4%		11%		6%		0%		2%			
MMF, Pred, Tac	55%		56%		65%		100%		50%			
Tac, Pred, Aza	13%		11%		18%		0%		12%			

Tac only	9%		0%		6%		0%		12%			
Tac, MMF	9%		0%		0%		0%		14%			
Pred, CsA, Aza	3%		0%		6%		0%		2%			
Tac, Pred	6%		22%		0%		0%		6%			
Tac, other	1%		0%		0%		0%		2%			
Anti-HLA antibodies at index bx		78		9		18		2		49	0.000	0.000
No anti-bodies	60%		0%		89%		100%		60%			
anti-HLA, non donor-specific	17%		0%		11%		0%		22%			
donor-specific	23%		100%		0%		0%		18%			

Mean GFR at index bx (if not on HD); ml/min	26 ± 13	66	21 ± 7	7	28 ± 15	12	24 ± 11	3	27 ± 14	44	n.s.	n.s.
HD at index bx	19%	81	22%	9	33%	18	0%	3	14%	51	n.s.	n.s.
Mean GFR at follow-up (if not graft loss); ml/min	40 ± 18	53	40 ± 19	3	38 ± 16	16	58 ± 21	2	40 ± 19	32	n.s.	n.s.
Graft loss	31%	81	44%	9	11%	18	33%	3	35%	51	n.s.	0.073
Drop in platelets at index bx	33%	80	22%	9	33%	18	50%	2	33%	51	n.s.	n.s.
Schisto-cytes at index bx	18%	67	11%	9	27%	11	100%	2	13%	45	0.037	n.s.
Signs of systemic TMA at index bx	18%	80	11%	9	17%	18	50%	2	18%	51	n.s.	n.s.

Low serum complement at index bx	33%	18 (unknown/not tested in 78%)	50%	2	0%	2	0%	1	38%	13	n.s.	n.s.
Significant hypertension at index bx	7%	81	0%	9	6%	18	33%	3	8%	51	n.s.	n.s.
High CNI level at index bx	33%	80	11%	9	94%	18	0%	2	16%	51	0.000	0.000
CMV-viremia at index bx	5%	65	11%	9	0%	8	0%	2	4%	46	n.s.	n.s.

Percentage may not sum to 100 due to rounding

¶Total number of valid observations may vary from total number of cases within respective group due to missing values or as variable does not apply to all patients

*Comparison between all groups; † comparison between ABMR- and CNI-related TMA

Tx, transplantation; bx, biopsy; MMF, mycophenolate mofetil; Pred, Prednisolone; CsA, Cyclosporin; Tc, Tacrolimus; Aza, Azathioprine; HD, hemodialysis; CNI, calcineurin inhibitor; IQR, interquartile range

Table 3: Main histological diagnosis in index-biopsies as well as microvascular inflammation, C4d- and DSA-status

		Index-biopsies in Assumed main etiological groups			
Main histological diagnosis	**All index-biopsies (n=81)**	**ABMR-related TMA (n=9)**	**CNI-related TMA (n=18)**	**other assumed cause of TMA (n=3)**	**cause of TMA unclear (n=51)**
Mean MVI-score¶	0.73 ± 1.3	1.2 ± 1.5	0.17 ± 0.5	0	0.88 ± 1.4
C4d positive#, n	13/79	6/9	1 (ABOi)/18	0/3	6 (of which 2 ABOi)/49
TMA as main diagnosis*; % (n)	77 (62)	(5) §	(18)	(3)	(36)
TCMR Banff I or II; % (n)	2.5 (2)	0	0	0	(2)
Banff III; % (n)	3.7 (3)	(1)	0	0	(2)
Histological features in keeping with ABMR†; % (n)	15 (12)	(3)	0	0	(9)
Other diagnosis‡; % (n)	2.5 (2)	0	0	0	(2)
DSA positive at index-bx, n**	18/78	9/9	0/18	0/2 (DSA status unclear in 1)	9/49

Percentage may not sum to 100 due to rounding
CNI, calcineurin inhibitor; TCMR, T-cell-mediated rejection; ABMR, Antibody mediated rejection; MVI, microvascular inflammation; DSA, donor-specific antibodies
*11 patients with borderline-changes in the background; † either g+ptc>0 and C4d>1 or g+ptc>1. In 4 patient there was evidence of chronic ABMR with Banff lesion score cg>1a ;
‡ acute pyelonephritis and histiocytic glomerulopathy of uncertain cause, respectively; § all C4d>1
¶ Microvascular inflammation Banff ptc-score+Banff g-score; not significantly different between groups (p=0.052)
# C4d positive=Banff C4d-score >1; significantly different between groups (p=0.002)
** significantly different between groups (p<0.000)

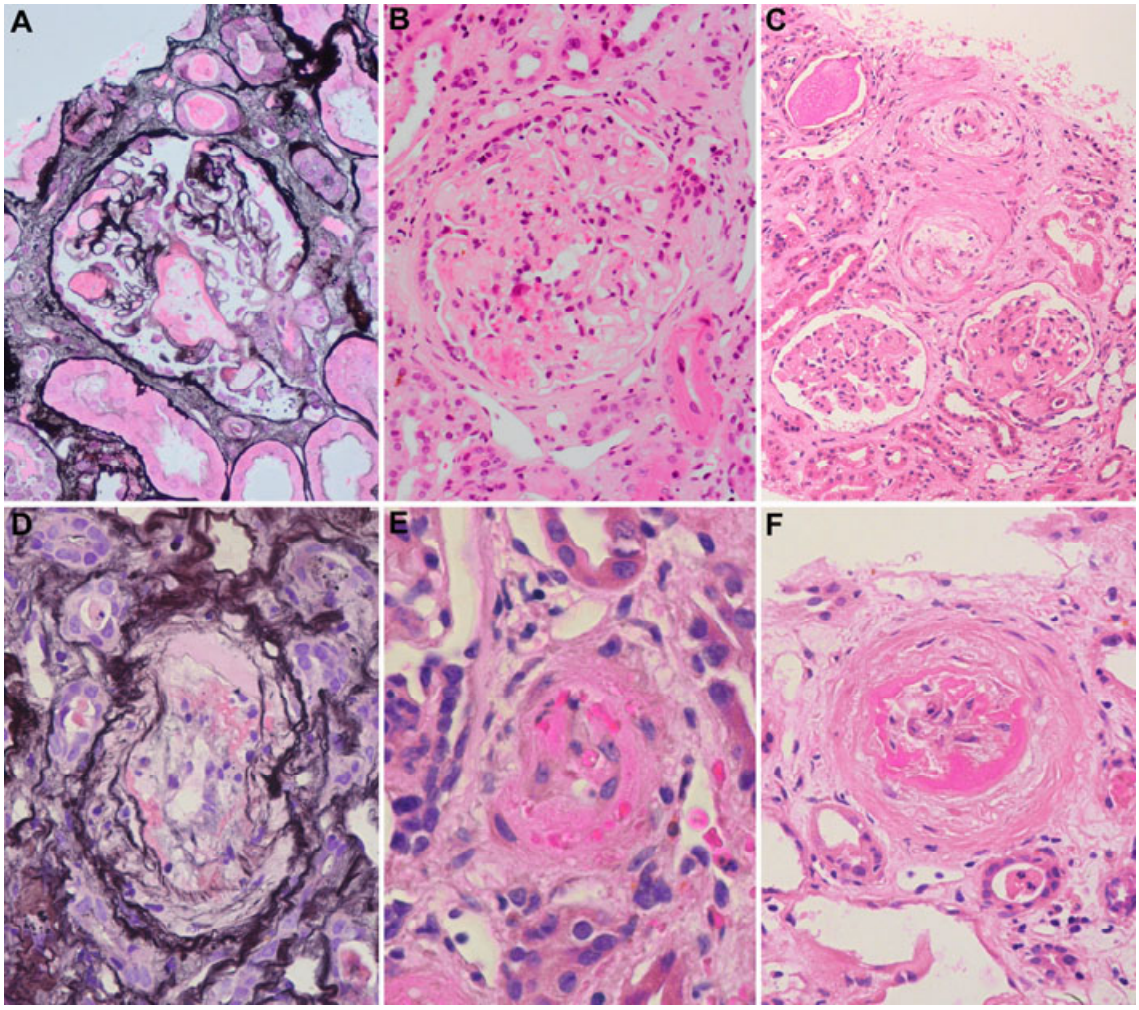
Table 4: Histological features of TMA for all biopsies with TMA and compared between TMA-groups

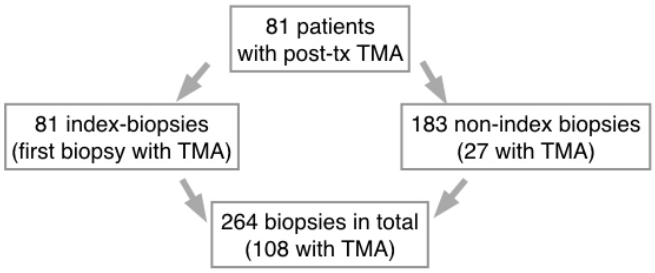
			Assumed main etiological groups									
Histological features of TMA	**All TMA-biopsies (n=108)**	total number of valid observations¶	**ABMR-related TMA (n=13)**	total number of valid observations¶	**CNI-related TMA (n=23)**	total number of valid observations¶	**other assumed cause of TMA (n=4)**	total number of valid observations¶	**cause of TMA unclear (n=68)**	total number of valid observations¶	**p-value***	**p-value†**
TMA present in glomeruli	75%	108	85%	13	70%	23	100%	4	74%	68	n.s.	n.s.
Extent of TMA in glomeruli; segmental/global, if TMA present in glomeruli	69%/ 31%	80	45%/ 55%	11	93%/ 7%	15	50%/ 50%	4	68%/ 32%	50	0.036	0.021
Median proportion glomeruli affected, if TMA present in glomeruli (IQR)	11% (6-29)	80	13% (8-59)	11	11% (8-16)	15	26% (12-37)	4	9% (6-17)	50	n.s.	n.s.
Presence of extra-glomerular TMA	58%	104	54%	13	55%	22	75%	4	58%	65	n.s.	n.s.

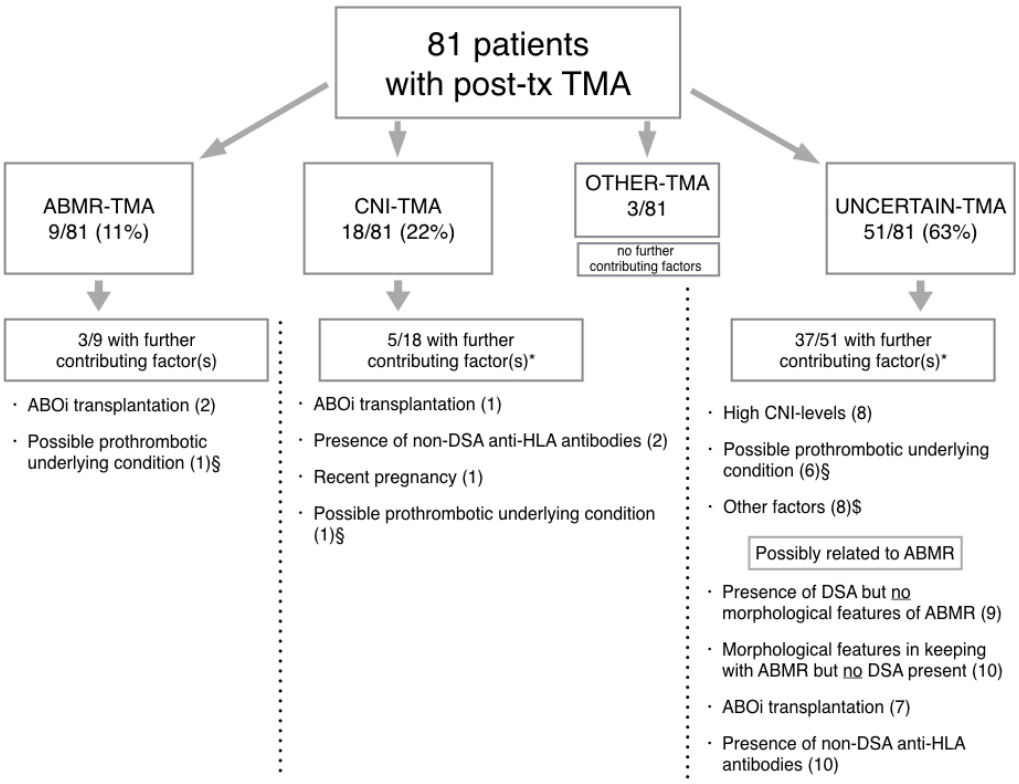
TMA present in arterioles	53%	107	54%	13	55%	22	75%	4	51%	68	n.s.	n.s.
TMA present in arteries	9%	105	0%	13	17%	23	0%	4	8%	65	n.s.	n.s.
Glomerular double contours present	10%	105	23%	13	0%	22	0%	4	12%	66	n.s.	0.044
Mesangiolysis present	15%	108	39%	13	4%	23	0%	4	15%	68	0.055	0.016
Fragmented RBCs in glomeruli present	10%	108	23%	13	0%	23	0%	4	12%	68	n.s.	0.04
Glomerular collape present	31%	105	46%	13	29%	21	25%	4	30%	67	n.s.	n.s.
Bloodless glomeruli present	32%	105	31%	13	29%	21	0%	4	36%	67	n.s.	n.s.
Intimal oedema in arteries/ arterioles present	47%	104	54%	13	36%	22	50%	4	49%	65	n.s.	n.s.

Fragmented RBCs in arteries/ arterioles present	24%	105	23%	13	13%	23	25%	4	28%	65	n.s.	n.s.
Intramural fibrin in arteries/ arterioles present	34 %	106	15%	13	35%	23	75%	4	35%	66	n.s.	n.s.
Fibrous intimal thickening in arteries present	11 %	105	15%	13	9%	23	0%	3	12%	66	n.s.	n.s.

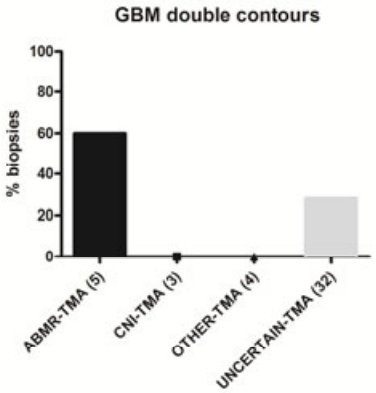
Percentage may not sum to 100 due to rounding
¶Total number of valid observations may vary from total number of cases within respective group due to missing values or as variable does not apply to all cases. In total, TMA was present in 108 biopsies but in one follow up biopsy with TMA no histological details were available.
*Comparison between all groups; † comparison between ABMR- and CNI-related TMA
TMA, thrombotic microangiopathy; ABMR, antibody mediated rejection; IQR, interquartile range

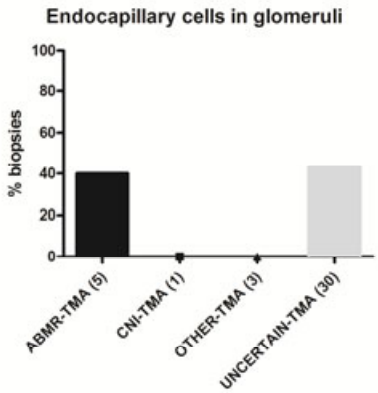


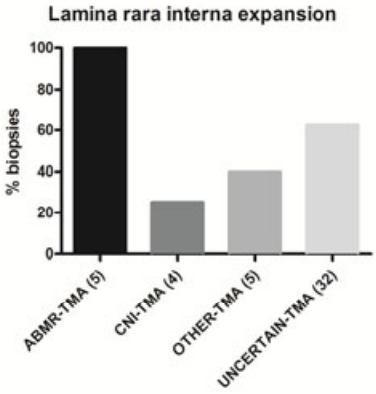


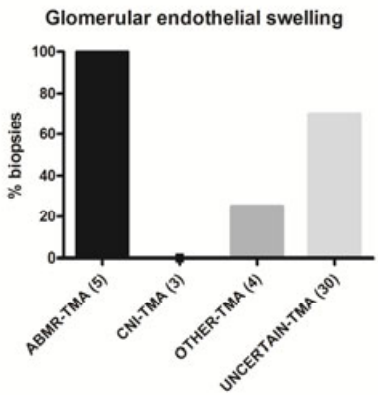


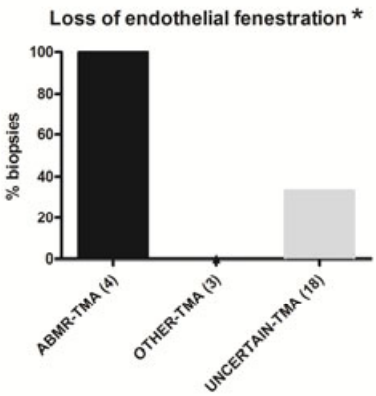
Ultrastructural changes in glomeruli











Ultrastructural changes in peritubular capillaries